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Nest structure, associated parasites and morphology of mature larvae of two European species of *Pseudoanthidium* Friese, 1898 (Hymenoptera, Megachilidae)

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Abstract

The bee genus *Pseudoanthidium* is represented by nine species in Europe. Of these nine species, *Pseudoanthidium nanum* is the most widespread, occurs mainly in xerothermic open habitats and creates nests in various cavity types. In this study, we provide information on the nest structure of this species in reed stalks and oak galls and about its parasitic species. We provide the first report of *P. nanum* as a host of *Xylophrurus augustus* (Ichneumonidae). The biology of the much rarer related species *Pseudoanthidium tenellum* is described here for the first time. This species occurs in terrestrial reed beds and wet meadows with the presence of reed galls and flowering plants in the family Asteraceae and is rare throughout its entire distribution area. This species nests inside reed galls induced by *Lipara* frit flies, and the nest structure is very similar to that of *P. nanum*. We report new parasitic species of this bee, namely, the cuckoo bee *Stelis punctulatissima*, the predator-inquiline *Gasteruption nigrescens* and two parasitoids, *Leucospis biguetina* and *Miltogramma punctata*. This bee collects pollen mainly from wetland plants in *Bidens* and *Pulicaria*. We also describe mature larvae of both species. The larvae do not differ greatly from one another; only the shape of mandibles and sclerotisation of mouthparts are slightly different. Further research should address the ecological requirements of *P. tenellum*, a poorly understood reed gall inquiline.

Keywords

Andricus, Megachilidae, oak gall, plant stem, *Pseudoanthidium*, reed bed, reed gall, wetland

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Introduction

The bee genus *Pseudoanthidium* Friese is a genus of small bees in the tribe Anthidiini, family Megachilidae, comprising 64 species worldwide (Michener 2007). This genus is divided into 12 subgenera, of which the subgenus *Pseudoanthidium* has the most species (Litman et al. 2016, 2021). In total, around 20 species are known from the western part of the Palaearctic biogeographic region, including nine species that occur in Europe (Kuhlmann et al. 2021; Litman et al. 2021). Of these nine species, *Pseudoanthidium nanum* Mocsary (syn. *P. lituratum* (Panzer)) is the most widespread, occurring in southern and central Europe, Russia and the Middle East (Banaszak and Romasenko 1998; Westrich 2018; Lhomme et al. 2020; Kuhlmann et al. 2021; Litman et al. 2021), and was recently introduced into several parts of the USA (Portman et al. 2019). This species prefers steppic formations and similar open habitats (Macek et al. 2010; Westrich 2018). The other species are much rarer: *Pseudoanthidium alpinum* Morawitz and *Pseudoanthidium tenellum* Mocsáry are rare species distributed in several countries of southern Europe and reach their northern distribution borders in central Europe – Austria, Germany, Hungary and Slovakia (Westrich 2018; Kuhlmann et al. 2021), and *Pseudoanthidium scapulare* Latreille is present in southwestern Europe and North Africa (Scheuchl and Willner 2016; Kuhlmann et al. 2021). *Pseudoanthidium stigmaticorne* (Dours) occurs in south Europe, North Africa, and the Middle East and *Pseudoanthidium canariense* (Mavromoustakis) on Canary Islands (Kuhlmann et al. 2021; Litman et al. 2021). Recently, Litman et al. (2021) described two new species that occur in the Middle East and worked on the taxonomy of the subgenus.

Multiple authors have studied the biology of *P. nanum* (Ferton 1908; Enslin 1925; Bischoff 1927; Micheli 1934; Grandi 1961; Schremmer 1985), and their results have been summarized in major European bee monographs (Banaszak and Romasenko 1998; Macek et al. 2010; Scheuchl and Willner 2016; Westrich 2018). This species creates its nests in various types of cavities in dead wood, plant stems (see Westrich (2018) for a list of plant species, in the stems of which the nests were recorded) and oak galls (Banaszak and Romasenko 1998). Bogusch et al. (2015) reported a specimen obtained from reed galls, but the specimen was probably hiding only inside, not nesting. Multiple parasitic species were associated with nests of this species – three species of cuckoo bee genus *Stelis* Panzer (*Stelis ornatula* (Klug), *S. punctulatissima* (Kirby) and *S. signata* (Latreille)), one sapygid wasp (*Sapyga quinquepunctata* (Fabricius)), six species of cuckoo wasps (Banaszak and Romasenko 1998; Wiesbauer et al. 2020), and six genera of chalcid wasps (Banaszak and Romasenko 1998; Scheuchl and Willner 2016). Grandi (1961) and Banaszak and Romasenko (1998) described the nest structure, Martynova (2020) described the structure of the cocoon of parasitic *Chrysis interjecta* Buysson in the nest of *P. nanum*, and Romasenko (1995) and Banaszak and Romasenko (1998) described the morphology of mature larvae. The species is oligolectic on Asteraceae (Banaszak and Romasenko 1998; Scheuchl and Willner 2016; Westrich 2018; Litman et al. 2021). Regarding *P. tenellum*, previous reports have mentioned only that it is oligolectic on Asteraceae and have provided distribution notes, but the

biology of this species has never been studied in detail (Banaszak and Romasenko 1998; Scheuchl and Willner 2016; Westrich 2018; Litman et al. 2021). Only Astapenková et al. (2017) recorded this species in cigar galls caused by *Lipara* Meigen frit flies (Diptera: Chloropidae) on common reeds, and Bogusch et al. (2020) reported the habitat requirements of this species.

We recorded specimens of *P. tenellum* in wetland habitats in Hungary and Slovakia and reared several of them from cigar galls induced by frit flies in the genus *Lipara* Meigen. We found the nests of this species inside the galls, and thus, we can provide information on habitat preferences, nest structure, parasites associated with *P. tenellum*, and the first description of its mature larvae. These records were compared with our records of nests of *P. nanum*, which we report from trap nests made of reed stalks and goldenrod stems and from oak galls of cynipid wasps.

Materials and methods

The studies of wetland fauna of Hungary and Slovakia were conducted mainly in Kiskunság National Park in central and southern Hungary in 2011–2018 and in the Danube Valley in southern Slovakia and northern Hungary in 2015–2021. The bees were captured using an entomological net, while in selected locations, reed galls were collected in winter (January – March) in numbers of 200–500 per locality and in smaller numbers in summer (June – July). The insects and other invertebrates were reared in special rearing bags, as described by Heneberg et al. (2014) and Bogusch et al. (2015). Only galls older than one year (greyish or darker in appearance, usually without leaves and with the apex broken) were collected because of our focus on cavity-nesting Hymenoptera (bees and wasps) instead of the *Lipara* spp. (inducing the galls) or their parasitoids. In the first years of the study (2015), we collected at least 500 reed galls at each sampling site, of which 200 were longitudinally cut, their contents were analysed, and the rest were allowed to rear (Bogusch et al. 2015; Astapenková et al. 2017). In 2017–2021, all collected galls were longitudinally cut to find nests of *P. tenellum*.

The trap nests were located in wetland and steppic parts of selected localities in the Czech Republic, Slovakia and Hungary, in numbers of 10 + 10 in 2017–2018. Each trap nest consisted of ten reed stalks and ten goldenrod stems fixed together by tape. The nests were placed on a bamboo stick 50–70 cm above the ground. They were installed in winter and early spring (February – April) and collected in autumn and winter (October – January). All reed stalks and goldenrod stems from all trap nests were longitudinally cut, and their inner contents were studied in the same way as in reed galls.

The oak galls of various species of the genus *Andricus* Hartig (Hymenoptera: Cynipidae) were collected in selected locations in the Czech Republic, Slovakia, Hungary, Austria, Croatia and Italy in 2017–2019 in winter months (December – March). All galls from one locality were placed into a plastic container, and reared insects were collected and studied. In 2018 and 2019, oak galls of *Andricus kollari* (Hartig) from four localities in the Czech Republic and two in Hungary were collected and cut to study their inner contents.

In the longitudinally cut reed galls, trap nests, and cut oak galls, we studied the material of the walls separating the brood cells (henceforth termed partitions) and the closing plugs at the top of each nest (henceforth termed closures), the structure and number of brood cells, and the morphology and colouration of larvae and pupae. In the descriptions, "first cell" denotes the bottom, i.e., first-built cell of the nest. The "last cell" denotes the uppermost cell, i.e., the one nearest to the nest entrance. When the larvae were in cocoons, we removed them from the cocoons but left the others inside. For each species, we first attempted to rear the adults. For nests containing more than three larvae, we conserved part of the brood for morphological studies. To rear the larvae, the living larvae were removed from the nests, placed in Eppendorf 1.5 ml microtubes, plugged with cotton wool, left at room temperature with ambient moisture, and reared similarly as described by Astapenková et al. (2017). The adults usually hatched within three to four weeks after pupation, after which they were fixed, similar to larvae, i.e., in 96% ethanol. The obtained material was identified by the first author. Representative specimens (including the nests of each species) are available in the collections of Petr Bogusch (University of Hradec Králové, Czech Republic).

We documented the representative part of the nests using a digital camera (photographs of entire nests) and a macrophotographing apparatus consisting of a macrocamera Canon attached to a stereomicroscope (brood cells and entire larvae). For the detailed photographs and photographs of morphological characters for the drawings, we used Keyence VHX digital microscope with camera and stacking software. We took photos of living larvae as well as the larvae fixed in Pampel solution (30 volumes of distilled water, 15 volumes of 96% ethanol, 6 volumes of formaldehyde and 4 volumes of glacial acetic acid) as described by Švácha and Danilevsky (1987). To describe the morphology of larval specimens, we transferred representative larvae into Pampel solution, while other larvae from the same nests were allowed to develop to adults, in order to identify them to species. After we took photographs of the intact larvae, we focused on their sclerotized parts. For this purpose, we placed the larvae into a 10% solution of hot (60 °C) potassium hydroxide for 12 hours to clear all parts of the body except the integument. Then, we coloured the integument in 5% chlorazol black E (Sigma Aldrich) for two seconds and moved the specimens into 96% ethanol for conservation. To observe the identifiable features, we placed the integument into glycerol and separately observed the head, mouthparts, spiracles and other important parts of the integument under a light microscope. We used the same specimens for the study of small structures such as setae, sensillae or mouthparts. We drew figures of (1) the head with a focus on the clypeus, labrum, maxillae, and labium; (2) the mandibles from the anterior view; and (3) the spiracles of larvae of each species.

Pollen samples were obtained from brood cells of selected nests from three localities. We collected all pollen from a selected brood cell in all cases. The pollen was stored in 75% ethanol. Then it was acetolyzed according to Faegri and Iversen (1989) and coloured with Safranin. Pollen grains were identified to taxa or pollen types (groups of taxa with morphologically unidentifiable pollen grains). Taxonomic identifications followed Punt and Hoen (2009) and Beug (2004).

Results

Records of *Pseudoanthidium tenellum* in Hungary and Slovakia

We recorded *P. tenellum* in seven localities in Hungary and two in southern Slovakia. All localities were terrestrial reed beds rich in bee and wasp fauna. The distribution of the localities is shown in Fig. 1. Detailed locality information includes the following.

Hungary centr., Dunatetetlén env., Bödi-Szék salt marsh, 46.7789317°N, 19.1408481°E, 8.VI.2013, 1 m* captured by net; Iszák, reed bed near Kolon-tó, 46.8033278°N, 19.3292419°E, 2.III.2015, 2 m*m* reared from reed galls of *Lipara lucens*; Sándorfalva env., reed margin near the road, 46.3548172°N, 20.0697903°E, 4.III.2015, 1 m* reared from reed galls of *L. lucens*, all P. Bogusch lgt.; ditto, 9.X.2021, two nests with brood, P. Heneberg lgt.; Hungary bor., Naszály, reed bed near pond reserve, 47.7030061°N, 18.2707742°E, 10.VII.2015, 1 f* captured sitting on reed gall; P. Bogusch lgt.; Hungary mer., Szeged env., terrestrial reed bed/wetland meadow ecotone, 46.2502361°N, 20.0460275°E, 10.II.2018, 2 f*f* reared from reed galls of *L. lucens*, 9.X.2021, eight nests with larvae in reed galls of *L. lucens*, all P. Heneberg lgt.; Hungary bor., Mocsa, wetland meadow, 47.6733947°N, 18.1936894°E, 20.VII.2021, two nests with larvae in reed galls of *L. lucens*, P. Bogusch lgt.; Hungary occ., Buzsák, terrestrial reed bed, 46.6642775°N, 17.5716547°E, 10.X.2021, three nests with larvae in reed galls of *L. lucens*; Hungary bor., Pákozd env., reed margin of Velencei-tó lake, 47.2152767°N, 18.5716825°E, 10.X.2021, one nest with larvae in reed galls of *L. lucens*, P. Heneberg lgt., all P. Bogusch det. & coll.

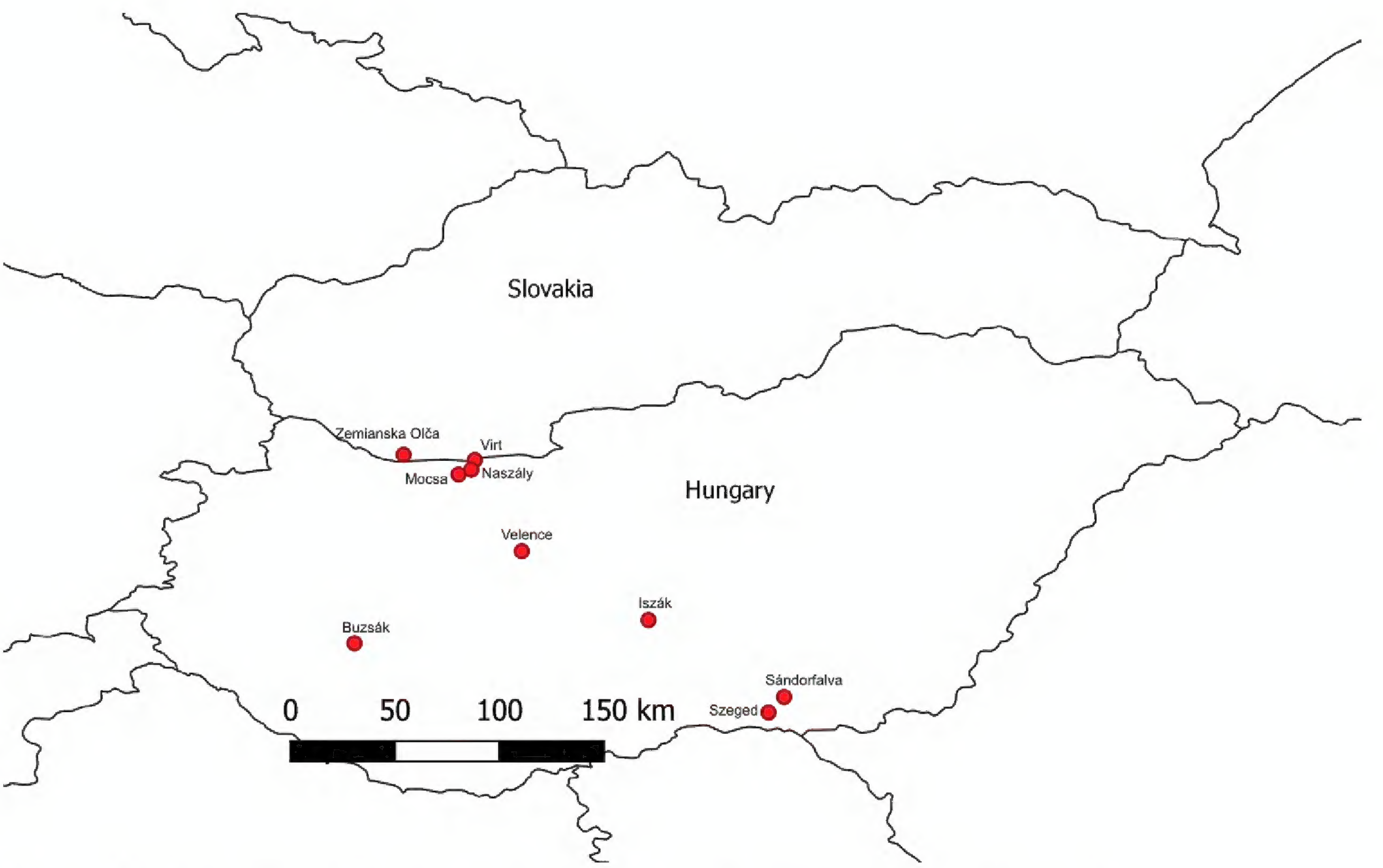

Figure 1. Map with localities of *Pseudoanthidium tenellum* in Slovakia and Hungary.

Figure 2. Nests of *Pseudoanthidium tenellum* inside a reed gall **A, B** entire gall with nest **C** detail on a brood cell with faeces, and **D** cocoon.

Slovakia mer., Marcelová-Virt env., 47.7600492°N, 18.2929869°E, terrestrial reed bed, 14.I.2016, 1 f* reared from reed galls of *L. lucens*, 19.I.2017, three nests with larvae in reed galls of *L. lucens*, 21.VII.2021, one nest with brood, Zemianska Olča env., terrestrial reed bed, 47.7915264°N, 17.8650603°E, 17.I.2017, two nests with larvae in reed galls of *L. lucens*, all P. Bogusch lgt., det. & coll.

Nest structure of *Pseudoanthidium tenellum* in reed galls

We collected 24 nests of *P. tenellum* from nine localities – seven localities in Hungary and two localities in Slovakia. The nests comprised 2–5 brood cells (median 3; mean 2.7 ± 0.5 cells per nest; n = 24 nests). The inner space of the gall was completely filled with whitish or yellow plant fibres, and brood cells were placed inside this matter (Figs. 2A-C). The filling was the same inside the entire nest, and no other material was used for the construction of the closing plug, partitions between brood cells, etc. The cocoons of oval shape with a typical projection on the bottom were light brown (Fig. 2D), and their length was between 7.32 and 8.02 mm (median 7.77, mean

7.70 ± 0.06 SD, n = 13) and maximal width was between 4.02 and 4.51 mm (median 4.35, mean 4.32 ± 0.04 SD, n = 13). Between the brood cells, there were faeces of yellow-orange colour (Fig. 2C).

Parasites associated with *Pseudoanthidium tenellum*

We recorded one larva of *Stelis punctulatissima* (Megachilidae), of which the adult hatched, in a nest from the Virt-Marcelová locality and one adult *S. punctulatissima* inside a cocoon in the nest from Mocsa. Two larvae of *Gasteruption nigrescens* Schletterer (Gasteruptiidae) were recorded in two nests from Szeged. Additionally, two nests from Szeged were completely parasitised by *Leucospis biguetina* Jurine (Leucospidae), three and four adults hatched. *Miltogramma punctata* Meigen (Diptera: Sarcophagidae) was recorded in three nests from Buzsák, two nests from Szeged and one from Sándorfalva in total number of 34 pupariums, from which 25 adults hatched. These records are the first published parasite associations with *P. tenellum*.

Pollen preferences of *Pseudoanthidium tenellum*

Because no information on pollen specialisation of *P. tenellum* occurs in the literature, we decided to do the analysis of pollen grains in brood cells of this species. Three nests, each from a different sampling site (Naszály, Virt and Zemianska Olča), contained pollen of plants in the family Asteraceae. The other plant families were represented only by single pollen grains, which were probably only accidentally introduced into the nest. The *Bidens tripartita* pollen type was the most abundant in all three nests, representing 73% of all pollen (53% in Virt, 68% in Zemianska Olča and 97% in Naszály). *Carduus* and *Arctium* were represented similarly in nests from Virt and Zemianska Olča (13% and 12%) and *Cirsium* with 2% in Naszály. Therefore, females of *P. tenellum* are oligolectic on Asteraceae and probably exploit wetland and ruderal species, which grow in places with the presence of reed galls (see Table 1). The pollen grains of *B. tripartita* from Virt are shown in Fig. 3.

Description of mature larva of *Pseudoanthidium tenellum*

Material examined

Two larvae from Szeged and one larva from Virt.

Diagnosis

The mature larva of *P. tenellum* is similar to the larva of *P. nanum*. In general, the body is thickened with small head and multiple setae on the surface. The main difference is in the shape of mandibles, which have blunter apical teeth, smaller antennal orbits and slightly different chaetotaxy. It corresponds in size with larva of *P. nanum*. The

Table 1. Proportions (in %) of pollen types in three samples from nests of *Pseudoanthidium tenellum*.

Pollen type	HU: Naszály	SK: Virt	SK: Zem. Olča	Mean
Bidens tripartita	97	53	68	73
Cirsium palustre	2	0	0	0.6
Arctium lappa	0	24	16	12
Carduus crispus	0	21	16	13

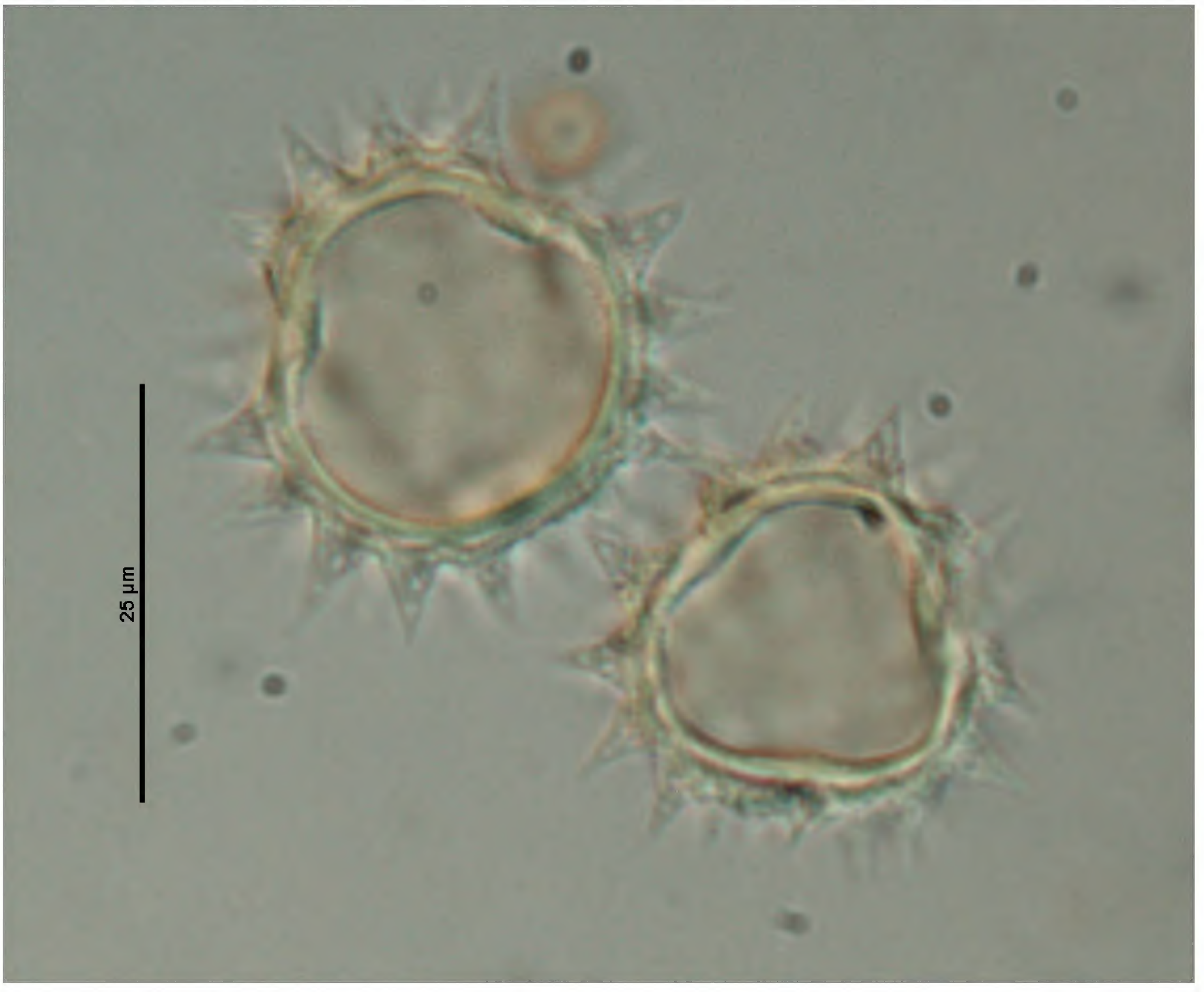

Figure 3. Pollen from brood cells of *Pseudoanthidium tenellum* with the dominance of the *Bidens* pollen type.

mandibles are light-brown coloured and less sclerotized than those of *P. nanum*. All studied larvae are very similar in general appearance and do not differ in the chaetotaxy and morphology.

Description

Body: Body length 5.8 – 7.1 mm (N = 3). Body vestiture without spicules, and with many thick, pale setae, tapering to fine points, arising from small but distinct alveoli. The distribution of setae is on the whole body, while the dorsal parts of body are more setose. Only mandibular apices, area around mandibular condyli, part of maxillae and labium and maxillar and labial palpi brownish coloured. Body form of postdefecating

larva fusiform, slightly dorsoventrally flattened, robust; all body segments of similar width (Fig. 4A). Dorsal tubercles present but ill-developed, only on metathorax and T1-T3, other body segments without body tubercles. Paired lateral tubercles on metathorax and T1-T8 but ill-visible, without any border. Body shape of predefecating larva in lateral outline with first abdominal segments having greatest diameter and outline tapering forward and very slightly backward from there. Abdominal segment 10 wide; anus positioned medially and transverse. Spiracles (Fig. 4D) light-brown, subequal in diameter; atrium globular, slightly wider than deep, projecting little above body wall, with rim; atrial opening diameter vs. peritreme width ratio 0.75; atrial inner surface with rows of wrinkles concentric with primary tracheal opening; primary tracheal opening with collar; subatrium short, with only four chambers of approximately equal size. Sex characters unknown.

Head: Head heart-shaped, small in relation to body size and ill-separated from prothorax; oriented in normal, hypognathous position relative to thorax. Setae long and sparse on upper part of head capsule; those of maxillary and labial apices large,

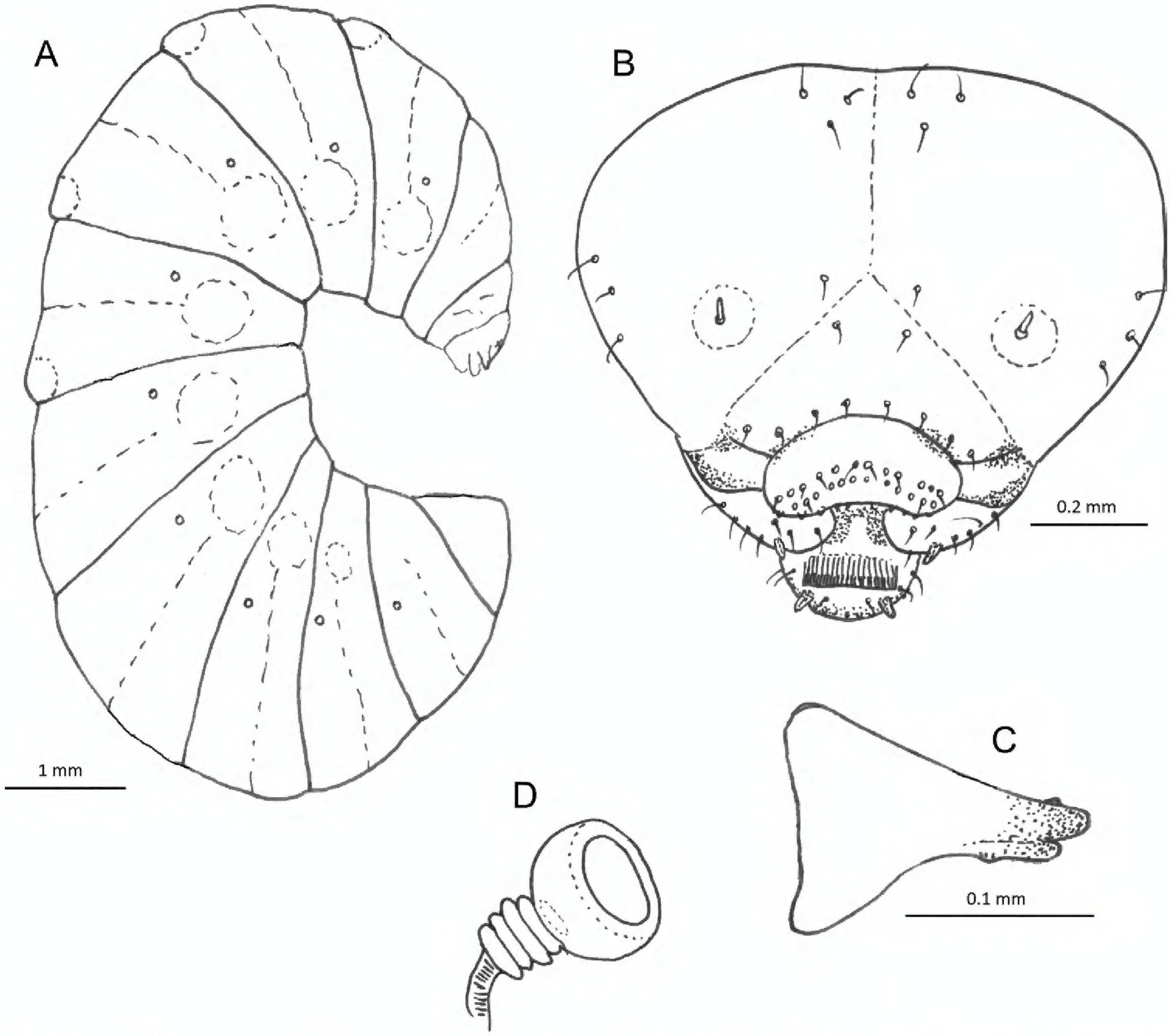

Figure 4. Mature larva of *Pseudoanthidium tenellum* **A** lateral view **B** head, frontal view **C** mandible, lateral view, and **D** spiracle.

straight, and conspicuous. Head capsule unpigmented except at points of articulations with mandibles; mandibles moderately pigmented except mandibular apices and areas of articulation with head capsule strongly pigmented; maxillary sclerites faintly pigmented; salivary lips projecting and pigmented; maxillary and labial palpi all uniformly moderately pigmented (Fig. 4B). Coronal ridge present, postoccipital ridges absent. Tentorium mostly absent because of impending ecdysis. Parietal bands absent. In lateral view, clypeus slightly convex but more than of *P. nanum*, projecting beyond frons, antenna arising from large but ill-developed prominence, and labrum extending beyond clypeus. Diameter of basal ring of antenna about ½ of the distance from closest point on ring to centre of anterior tentorial pit; antennal papilla only slightly pigmented, elongate, bearing two sensillae apically. Frontal area between antennae with only two setae on each side. Parietal region with many setae - three setae from pleurostomal ridge to front tentorial pit and several sensilae on the sides. Three setae on the top of parietal area. Clypeus wide with ill-developed basal and well-developed apical margin, four setae subapically on each side. Labrum C-shaped, distinctly emarginated apically in the middle, with two rows of more than ten setae and sensillae and a row of sensillae apically; labral sclerite not defined and only very poorly pigmented. Epipharynx simple without any visible spinulae. Mandible moderately robust; apex brownish pigmented, with two blunt apical teeth longest and two blunt lateral tubercles (Fig. 4C). Maxillary apex slightly bent mesad in frontal view, so that maxillary palpus subapical in position; cardo distinct, posterior end directed toward posterior tentorial pit; stipes weakly sclerotized; maxillary palpi elongate, probably more than four times basal diameters, both pigmented. Stipes with ten conspicuous setae, which are slightly larger than those on other parts of head. Maxillar palpus elongate, with three sensillae on the top. Labium not divided into prementum and postmentum; apex moderately narrow in frontal view. Four setae on both sides and three smaller on ventral surface. Salivary lips transverse, very wide and well visible, with inner surface bearing parallel longitudinal grooves; width of lips more than half of width of labium. Labial palpus elongated with three sensillae in middle.

Nest structure of *Pseudoanthidium nanum* in plant stems and oak galls

The nests of *P. nanum* in reed stalks and goldenrod stems from trap nests differed in several cases. More nests were recorded in trap nests made from reed stalks (132 of 156 nests in total) than in trap nests made from goldenrod stems (24 nests), although both were made available in similar quantities. The number of brood cells per nest was 2–17 (median 6; mean 6.6 ± 3.2 cells per nest; n = 156 nests). The number of brood cells in goldenrod stems was lower (range 2–12, median 5; mean 5.2 ± 2.3 cells per nest; n = 24 nests) than that in reed stalks (range 2–17, median 6; mean 6.7 ± 3.3 cells per nest; n = 132 nests). However, the number of brood cells was limited by the cavity length. The inner space of the cavities was filled by plant fibres usually of white or whitish colour; several times, the colour was light-brown, yellow, orange or reddish, and in several nests, the colour varied in the length of the nest, certainly according to the

matter used by the nesting female. The nest did not have any matter at the base or closing plug. The entire inner space was filled with plant fibres (Figs. 5A). Cocoons were located inside the filling; they were brown coloured and of the same shape as cocoons of *P. tenellum*. The only difference was the darker colour of the cocoons than those of *P. tenellum* (Fig. 5E). The size of the cocoons was similar to that of *P. tenellum*, length 7.02–8.41 mm (median 7.84; mean 7.8 ± 0.4 mm; n = 42 cocoons measured) and width 4.02–4.61 mm (median 4.32; mean 4.35 ± 0.3 mm; n = 42 cocoons measured).

The nests in oak galls were much smaller, and they contained 2–6 brood cells (median 3; mean 3.1 ± 1.2 cells per nest; n = 19 nests). The brood cells were located in the soft parenchymatic tissue between the central brood cell of *A. kollari* and the gall outer layer (Figs. 5B-D). They were usually oriented The basal parts of the cocoons were usually located near the centre of the cell. The cells were covered by plant fibres of usually whitish or light grey colour. The sizes of cocoons were similar to those in reed stalks and goldenrod stems: length 7.10–7.99 mm (median 7.65; mean 7.68 ± 0.40 mm; n = 12 cocoons) and width 4.09–4.53 mm (median 4.30; mean 4.33 ± 0.20 mm; n = 12 cocoons).

Figure 5. Nests of *Pseudoanthidium nanum* **A** nest in a twig of *Rosa* sp. **B, C** nests in oak galls of *Andricus kollari*, D – two cocoons in an oak gall, and E – cocoon.

Parasites associated with *Pseudoanthidium nanum*

We recorded one cuckoo bee, one cuckoo wasp, two species of chalcids and one ichneumonid in nests of *P. nanum*. *Stelis punctulatissima* (Megachilidae) was the most abundant species, recorded from 38 nests in reed stalks, while eight of these nests contained only the brood of *S. punctulatissima* (but the nest structure was certainly from *P. nanum*). This parasitic species was also reared from nine nests with *P. nanum* in oak galls (from the Czech Republic, Slovakia, Hungary and Italy) of *A. kollari* and from two nests in oak galls of *Andricus quercustozae* (Bosc) from Italy. *Chrysis interjecta* (Chrysididae) was found in seven nests in reed stalks from Hungary, in two oak galls of *A. kollari* (from Slovakia and Hungary) and in three oak galls of *A. quercustozae* (from Italy). *Melittobia acasta* (Walker) (Chalcidoidea: Eulophidae) was recorded in six nests in reed stalks, one in goldenrod stems and two in oak galls of *A. kollari*. *Eurytoma* sp. (Chalcidoidea, Eurytomidae) was recorded in two nests in reed stalks, and *Xylophrurus augustus* (Dalman) (Ichneumonidae) was recorded in two nests in reed stalks.

Description of mature larva of *Pseudoanthidium nanum*

Material examined

Ten larvae from Lanžhot, Břeclav-Pohansko, Kurdějov and Hodonín.

Diagnosis

The mature larva of *Pseudoanthidium nanum* is similar to larva of *P. tenellum*. In general, the body is thickened with small head and multiple setae on the surface. The main difference is in the shape of mandibles, which have blunt apical teeth, larger antennal orbits and slightly different chaetotaxy. It corresponds in size with larva of *P. tenellum*. The mandibles are brownish coloured and more sclerotized than that of *P. tenellum*. Both studied larvae are very similar in general appearance and do not differ in the chaetotaxy and morphology.

Description

Body: Body length 6.1 – 7.9 mm (N = 10). Body vestiture without spicules, and with many thick, pale setae, tapering to fine points, arising from small but distinct alveoli. The distribution of setae is on the whole body, while the dorsal parts of body are more setose. Only mandibular apices, area around mandibular condyli, part of maxillae and labium and maxillar and labial palpi brownish coloured. Body form of postdefecating larva fusiform, slightly dorsoventrally flattened, robust; body segments similarly wide on whole length (Fig. 6A). Dorsal body tubercles present but ill-developed only on metathorax and T1-T3, other body segments without body tubercles. Paired lateral tubercles present on metathorax and T1-T8 but ill-visible, not separated. Body shape

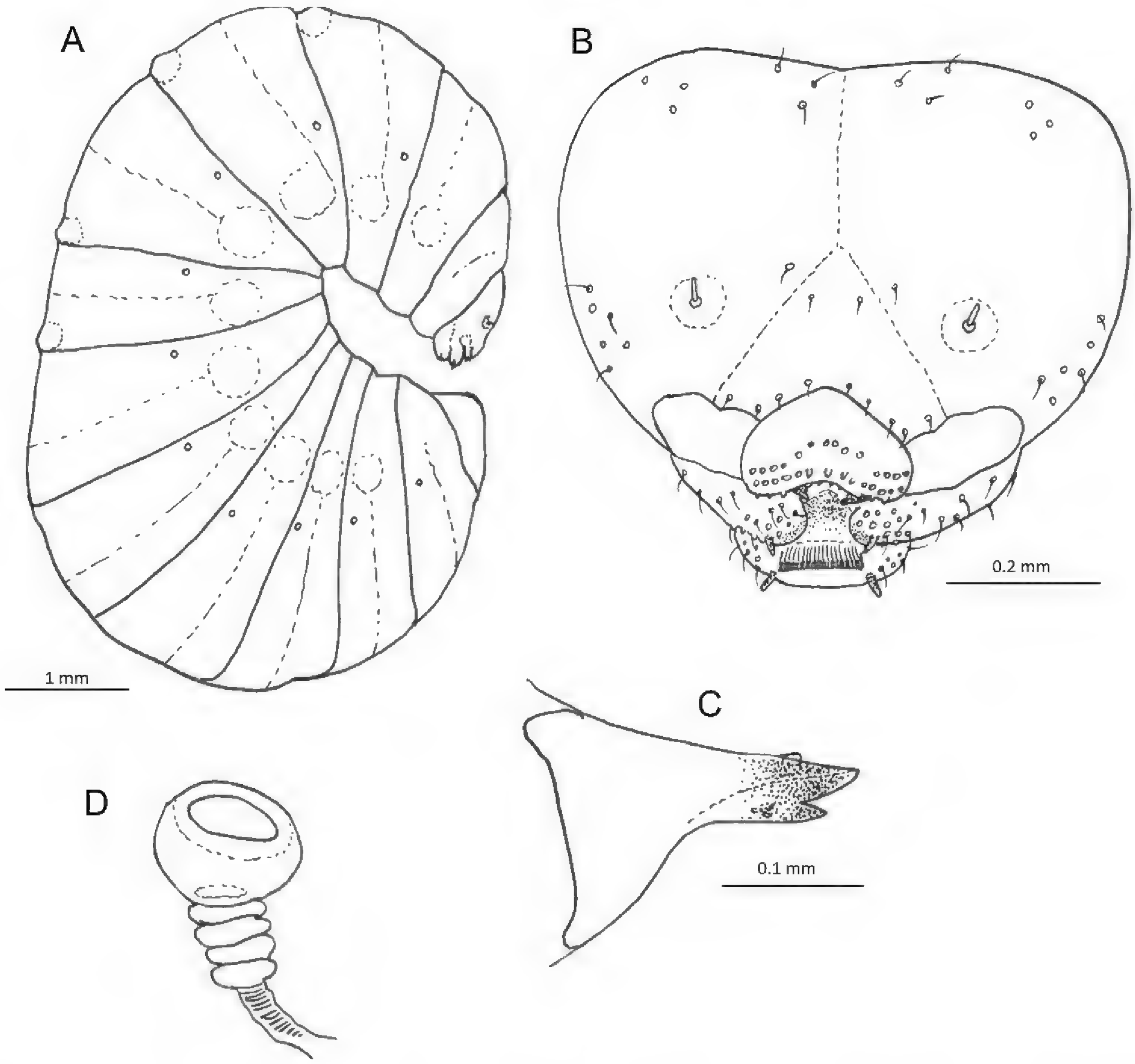

Figure 6. Mature larva of *Pseudoanthidium nanum* **A** lateral view **B** head, frontal view **C** mandible, lateral view, and **D** spiracle.

of predefecating larva in lateral outline with first abdominal segments having greatest diameter and outline tapering forward and very slightly backward from there. Abdominal segment 10 wide; anus positioned medially and transverse. Spiracles (Fig. 6D) light-brown, subequal in diameter; atrium globular, slightly wider than deep, projecting little above body wall, with rim; atrial opening diameter vs. peritreme width ratio 0.75; atrial inner surface with rows of wrinkles concentric with primary tracheal opening; primary tracheal opening with collar; subatrium short, with only four chambers of approximately equal size. Sex characters unknown.

Head: Head heart-shaped, small in relation to body size and ill-separated from prothorax; oriented in normal, hypognathous position relative to thorax. Setae long and sparse on upper part of head capsule; those of maxillary and labial apices large, straight and conspicuous. Head capsule unpigmented except at points of articulations with mandibles; mandibles moderately pigmented except mandibular apices and ar-

eas of articulation with head capsule strongly pigmented; maxillary sclerites faintly pigmented; salivary lips projecting and pigmented; maxillary and labial palpi all uniformly moderately pigmented (Fig. 6B). Coronal ridge present, postoccipital ridges absent. Tentorium mostly absent because of impending ecdysis. Parietal bands absent. In lateral view, clypeus only slightly convex, projecting beyond frons, antenna arising from large but ill-developed prominence, and labrum extending beyond clypeus. Diameter of basal ring of antenna similar to the distance from closest point on ring to center of anterior tentorial pit; antennal papilla only slightly pigmented, elongate, bearing two sensilla apically. Frontal area between antennae only with two setae on each side. Parietal region with four setae and four sensillae laterally near mandibular condyli and three setae and one sensilla on each side near the coronal ridge. Clypeus wide with ill-developed basal and well-developed apical margin, four setae subapically on sides. Labrum C-shaped, distinctly emarginated apically in the middle, with two rows of more than ten setae and similarly large sensillae on the surface and one row apically. Labral sclerite not defined and only very poorly pigmented. Epipharynx simple without visible spinules. Mandible moderately robust; darkly pigmented, with two apical teeth longest and sharp and with one subapical and one lateral tubercle (Fig. 6C). Maxillary apex slightly bent mesad in frontal view, so that maxillary palpus subapical in position; cardo distinct, posterior end directed toward posterior tentorial pit; stipes weakly sclerotized; maxillary palpi elongate, probably more than four times basal diameters, both pigmented, with three sensillae apically. Cardo with six, stipes with thirteen conspicuous setae, which are larger than setae on other parts of head. Labium not divided into prementum and postmentum; apex moderately narrow in frontal view. Three large setae on both sides and five smaller on ventral surface. Salivary lips transverse, very wide and well visible, with inner surface bearing parallel longitudinal grooves; width of lips more than half of width of labium. Labial palpus elongated with three sensillae in middle.

Discussion

Summarizing the previously published data on the nesting biology of *P. nanum*, we can obtain a good overview of most aspects of the nesting biology of this species. This bee uses various types of cavities for nesting, while those in wood and plant stems are the most preferred. In trap nests composed of reed stalks and goldenrod stems, this species was the second most numerous in all habitats, while it highly preferred nesting in reed stalks with an existing cavity. Comparing the wetland and steppic habitats, it was much more abundant in steppic habitats, where it was the most abundantly nesting bee species in a survey by Heneberg et al. (unpublished). However, we did not observe this species as nesting in reed galls, although many thousands of reed galls were analysed for this purpose (summarized by Astapenková et al. 2017 and Bogusch et al. 2020). *Pseudoanthidium nanum* certainly uses pre-existing cavities for its nesting, and it is likely unable to enter the gall, where the entrance is hidden (see Bogusch et al. 2020).

Reed galls have the entrance hidden among old leaves and nesting species must evolve specific strategies to get into, which is more complicated than in the case of open entrances of reed stalks. *Pseudoanthidium nanum* was also recorded as a very numerous species nesting in oak galls (P. Bogusch & P. Heneberg, pers. obs.). In oak galls of various species of cynipid wasps in the genus *Andricus*, *P. nanum* preferred galls of *A. kollari*, probably because of their high abundance, positions low above the ground, and large openings formed by reared cynipid wasps. Several nests were also in galls of *A. quercustozae* but none in those of *Andricus hungaricus*, probably because these galls do not stay on twigs but fall on the ground at the end of the season.

The structure of the nest reflects the cavity type. In linear cavities, all or most of the inner space is filled by plant fibres (see Fig. 5A), as reported by Grandi (1961), Banaszak and Romasenko (1998) and Westrich (2018). The brood cells are not separated, and there are only pollen balls of oval shape with eggs or larvae inside the plant fibre matter. In oak galls, brood cells are usually formed separately in cavities, from which the nesting female removed the parenchymatic tissue before (see Figs. 5B-D). This corresponds with the hypothesis that *P. nanum* is ecologically plastic and can utilize many types of cavities (Banaszak and Romasenko 1998; Westrich 2018).

Regarding *P. tenellum*, we recorded nests only in reed galls. This species occurs in near-natural terrestrial reed beds with meadows or semiruderal sites, where a high number of flowering plants (both in abundance and diversity) exists. However, this species is rare, and only unexpected findings at two localities during our survey of reed galls in Hungary (Astapenková et al. 2017) led us to study the biology of this species in detail. Certainly, it is a question, whether this species has the same natural history in its whole very large distribution area, or is restricted to wetland habitats with reed galls only in the westernmost part of its area of occupancy. Contrary to *P. nanum*, *P. tenellum* found a way to enter the reed gall and created nests in galls with hidden entrances. The nest can be identified at first sight, as plant fibres, with brood cells inside, which filled all or most of the hollow. This nest structure is very similar to that of *P. nanum* in linear cavities. The brood cells are not separated; there are only pollen partitions of oval shape with eggs or larvae inside the plant fibre matter. Contrary to *P. nanum*, whose nests in linear cavities frequently contain ten or more brood cells, the number of brood cells in nests of *P. tenellum* inside the limited space of reed gall is much lower.

Pseudoanthidium tenellum is certainly a species occurring predominantly in wetlands and reed beds. During our long-lasting surveys on bees in Hungary (since 1999), we recorded only the specimens listed in this study, and all originated from wetland localities. Many of the localities contain high proportions of salt in the ground and can thus be classified as inland salines. Westrich (2018) also reported the occurrence of this species in saline habitats, but the species also occurs in habitats with low concentrations of salt in the ground. The pollen obtained from nests also confirms this habitat requirement (described by Astapenková et al. 2017 and Bogusch et al. 2020) because most pollen in nests of this species was identified as the *Bidens* pollen type – pollen of the wetland plants of the genus *Bidens* according to Beug (2004). Litman et al. (2021) recorded *Limonium* as a host plant, but they explained that this plant was visited by a

single male only. More authors have reported steppic plants visited by this species, but they may serve as a source of nectar. We did not study the diet of *P. nanum*, Westrich (2018) cited mostly steppic (*Centaurea* and *Inula*) and ruderal plant species (*Solidago*) of the family Asteraceae.

Although no parasitic species associated with *P. tenellum* were recorded (perhaps because the biology of this species was previously unknown), we surprisingly identified one *Stelis punctulatissima* in a nest from Virt. This species is an unspecialized nest cleptoparasite of multiple genera of bees in the family Megachilidae (see Banaszak and Romasenko (1998) and Westrich (2018)). It was also occasionally reared from nests of *Hoplitis leucomelana* (Kirby) in reed galls (Bogusch et al. 2015). It was found in high numbers in nests of *P. nanum* both in reed stalks and cynipid galls (this study). Interestingly, *G. nigrescens* larvae inside two nests from Szeged (Hungary) were also found. This rare species of open habitats in central Europe (both xerothermic and wetland) is associated with *H. leucomelana* as the main and best-known host (Schmid-Egger and Saure 2010). However, the host spectrum could be broader because Bogusch et al. (2018) reported this species frequently parasitizing nests of *Heriades rubicola* Pérez, which is recently enlarging its area in central Europe towards the north. Finding this species in the nests of another megachilid bee, *P. tenellum*, is thus not surprising. Interestingly, larvae of *Gasteruption* usually eat out the entire inner space of the nest, and only a single larva survives in the nest of the host (see Parslow et al. 2020). However, in the case of *G. nigrescens* parasitising nests of *H. rubicola* and *P. tenellum*, the larvae usually remained in the brood cells and did not move between cells inside the cavity like larvae of this genus usually do (Bogusch et al 2018; Bogusch 2021). *Leucospis biguetina* is a parasitoid associated with many species of bees s. l. (Baur and Amiet 2000) nesting in cavities. Similar is the situation with the sarcophagid fly *M. punctata*, which also attacks nests of many species of bees and wasps, both nesting in soil and cavity (Westrich 2018). This species is widespread in Europe and has probably the broadest spectrum of hosts within the genus, of which all members are nest cleptoparasites of solitary bees (Whitmore et al. 2020). Thus, the findings of both species in nests of *P. tenellum* are not surprising, although *L. biguetina* is not very common species (Baur and Amiet 2000).

Regarding the parasites of *P. nanum*, we recorded those whose association with *P. nanum* is well known. The very rare species *Chrysis interjecta* was frequently recorded, usually in warmer regions south of Slovakia and in Hungary and Italy and only in oak galls. The only new host record was the finding of *Xylophrurus augustus* in nests of *P. nanum*. However, this fact is not surprising because this species is not specialized, and many species of bees, sawflies, and saproxylic beetles have been reported as hosts (for a review, see Yu et al. 2012).

The larva of *P. nanum* was described by Romasenko (1995), and our findings generally agree with the original description. It is a typical megachilid larva with a heart-shaped head, bidentate mandible, elongated antennal projection and integument with many short spinules. Larvae obtained from oak gall did not differ from those from reed stalks. Although the larvae of *P. tenellum* look very similar, we can find several differ-

ences between mature larvae of both species. The main difference is the blunt apical tooth on the mandible, in contrast to the sharp tooth of *P. nanum*, and less sclerotized and thus lighter coloured parts of the head. In general, it corresponds with the fact that both species are closely related, although they developed very different ecologies.

Pseudoanthidium nanum is a relatively common species of open habitats in central Europe. It usually occurs on forest-steppe grassland slopes, where it forms strong populations. Adults can be found easily on flowers of various plants in the family Asteraceae. They need shrubs and/or large and thick plant stems for their nesting or the presence of abandoned oak galls. They are common and numerous at suitable sites and many parasitic species have adapted to exploit nests of this bee species. *Pseudoanthidium tenellum* is a much rarer species in terrestrial reed beds and wet meadows with the presence of flowering plants (especially in the family Asteraceae) and reed galls. It is very rare and occurs in habitats that are near-natural and well preserved. In this study, we recorded the first four parasitic species associated with *P. tenellum* and described its nest structure, nesting biology and mature larva. Because the distribution and probably several aspects of the biology of this rare bee remain unknown, our study can serve as a starting point for future studies on this bee species, reed beds and reed galls.

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